



Beta-Lactam Antibiotics Induce Protein Expression Changes in *Neisseria gonorrhoeae* Revealed by a Proteomic Approach

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Introduction

Neisseria gonorrhoeae infection is a serious challenge for public health. The gonococcal resistance to extended-spectrum cephalosporins (ESCs) as well as treatment failures with ESCs have been increasingly reported in many countries globally. These increasing trends together with the limitation of drugs of choice lead gonorrhoea to become untreatable in the near future. In recent time, proteome analysis is a powerful tool that permits simultaneous investigation of thousands of proteins of different samples. Therefore, in order to reveal more understanding on the antibiotic effects, the physiological response of gonococci to ESCs as well as searching for potential protein for drug development, proteomic approach was adopted to investigate gonococcal protein expression under ESC stresses.

Materials and Methods

N. gonorrhoeae was grown on GC broth with or without 0.5x MIC of antibiotic. Proteins were extracted and subjected to separate using 13 cm pH 3–10 IPG strips and 12 % SDS-PAGE. Analysis for differences in protein expressions was performed with the ImageMaster 2D Platinum software tool. Protein spots of interest were excised from 2-DE gels and digested with trypsin. Protein identification was performed by peptide-mass fingerprinting using a MALDI-TOF mass spectrometer. Finally, peptide masses were searched using a Mascot server.

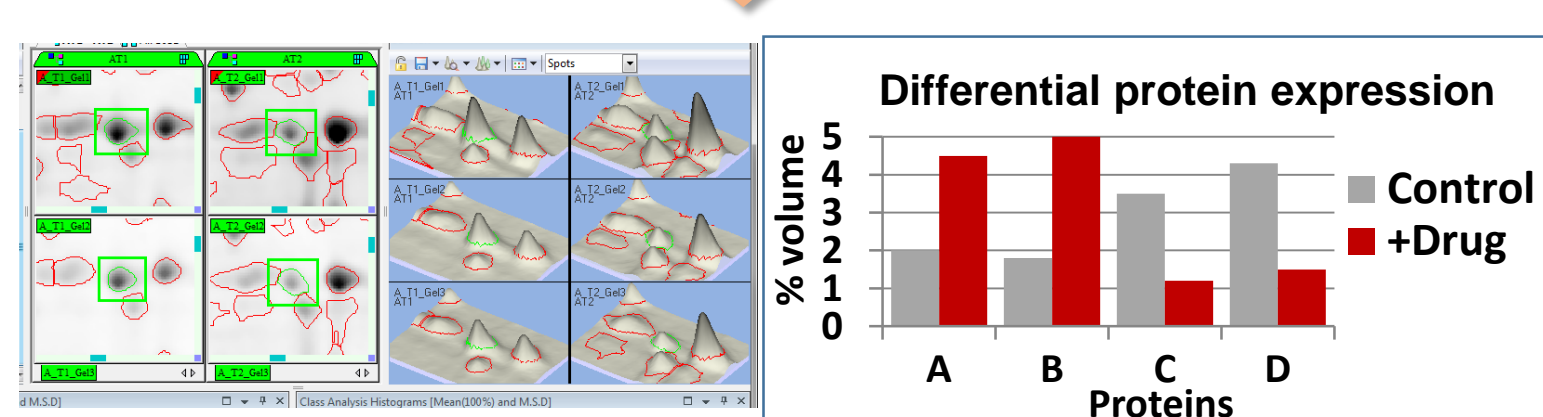
1. Cell preparation



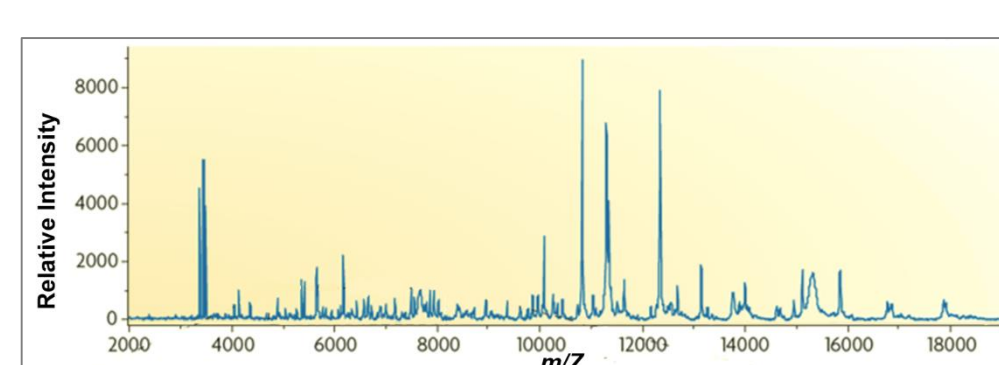
2. Proteomic analysis



3. Differential analysis



4. Protein identification



5. Database Searches

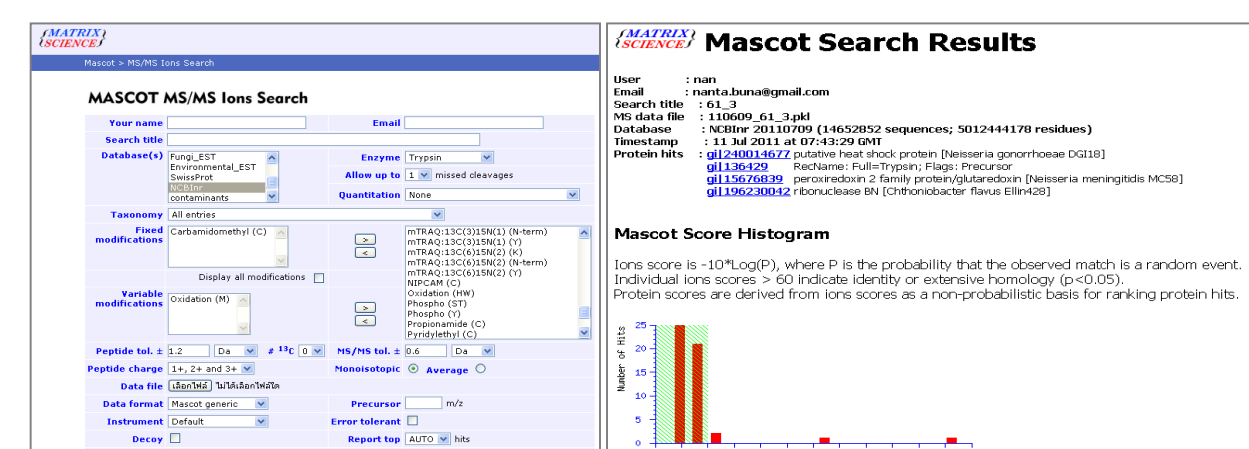


Figure 1 Experimental workflow of protein expression analysis of gonococci in response to ESC stress.

Results and Discussion

The whole-cell protein extract of *N. gonorrhoeae* reference strain was analyzed using the 3–10 range IPG strip and approximately 200 spots were visualized by colloidal Coomassie blue staining. The cellular responses of *N. gonorrhoeae* to sub-MIC of ESCs were investigated by comparing the protein expression profile of the ESC-treated culture to those of control culture. Differentially expressed proteins were selected and identified by MALDI-TOF MS. Results showed that 14 and 13 spot proteins were significantly altered expression following exposure to ceftriaxone and cefixime, respectively (Figure 2). Most of expressed proteins shared a similar expression pattern in response to ceftriaxone and cefixime. These results reflected similarities in antibiotic mechanisms of action.

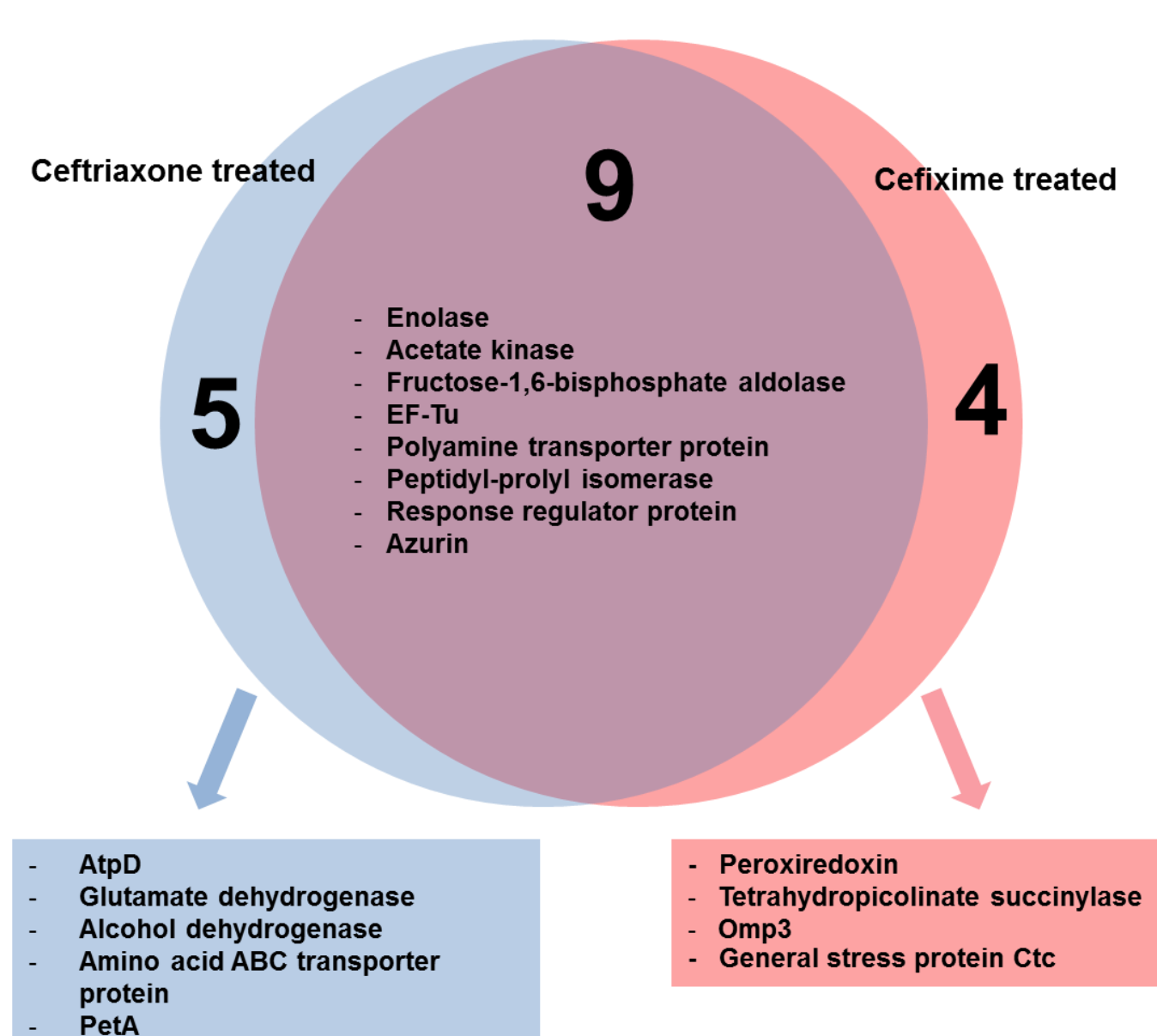


Figure 2. Venn diagram of number of differentially expressed proteins induced by ceftriaxone and cefixime

As seen in Figure 2, some proteins showed altered expression particularly for cefixime treatment. These results might support the previous suggestion in which cefixime is fluxed through gonococcal cell with different manner from ceftriaxone.

Then, all of the identified proteins were predicted subcellular localization and also classified the biological functions. As shown in Figure 3, although cephalosporin is antibiotic inhibiting cell wall synthesis, ESCs triggered proteins in a variety of cellular locations and also in a variety of biological functions, such as energy metabolism, transport system, cell envelope, stress response, virulent factor and etc. Proteins belonged to the energy metabolism and the nutrient transport system showed increased expression under ESC stress. These circumstances suggested to help gonococci to survive under antibiotic stress.

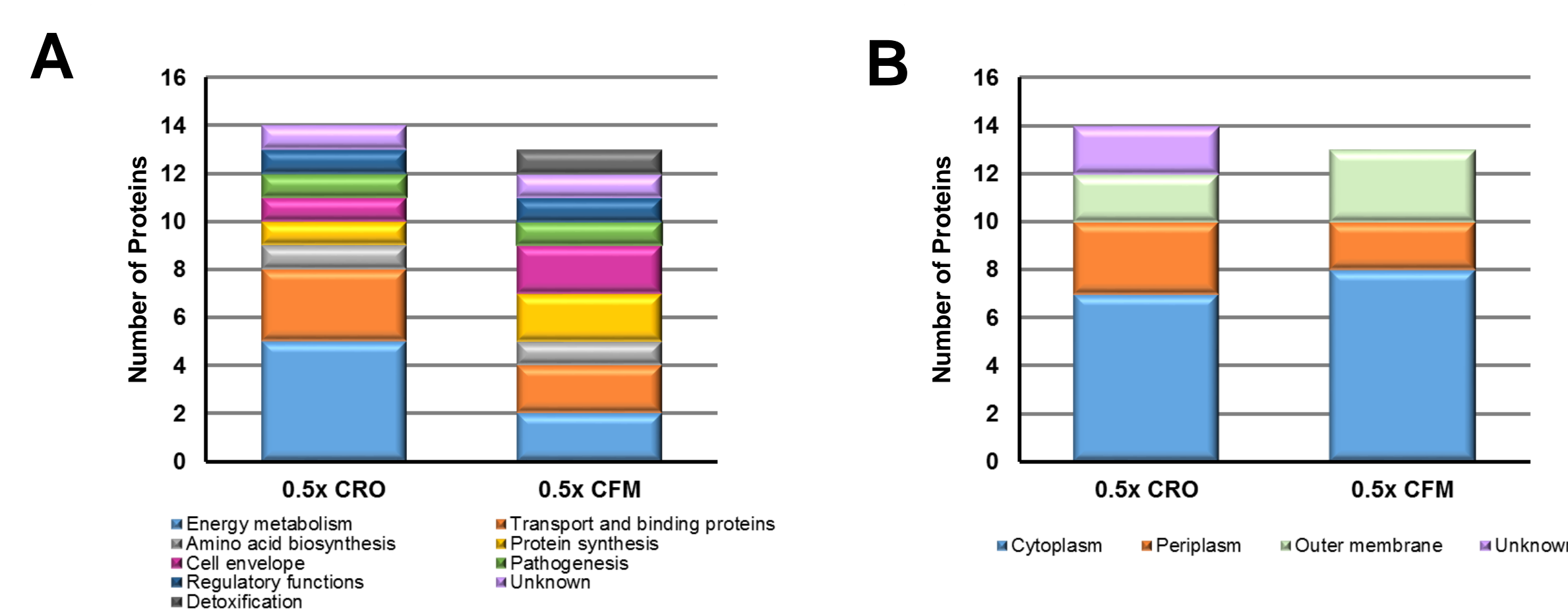


Figure 3. Distribution of biological functions (A) and the subcellular location (B) of proteins differentially expressed in response to ESC stresses.

Moreover, the up-regulation of azurin and peroxiredoxin may reflect the defense mechanism of gonococci in response to ESC inducing oxidative stress. Interestingly, our results showed that sub-inhibitory concentration of ESCs can enhance the expression of bacterial virulent factor that are azurin and PPIase. Azurin facilitates gonococci to survive within cervical epithelial cells while PPIase helps this microorganism to survive within in macrophage. The discovery of novel target or novel compound is needed to fight against gonococcal infection and gonococcal antibiotic resistance. Virulence factors is an attractive drug target for anti-infection. Thus, understanding on the their exact biological functions during infection , the response to ESC as well as drug development are needed for further research.

In conclusion, the present work might provide new insights into physiological adaptive networks of gonococci to antimicrobial agents and more understanding toward the mechanism of action, which subsequently may benefit for the further drug discovery of new antimicrobials to combat with resistant gonococci.

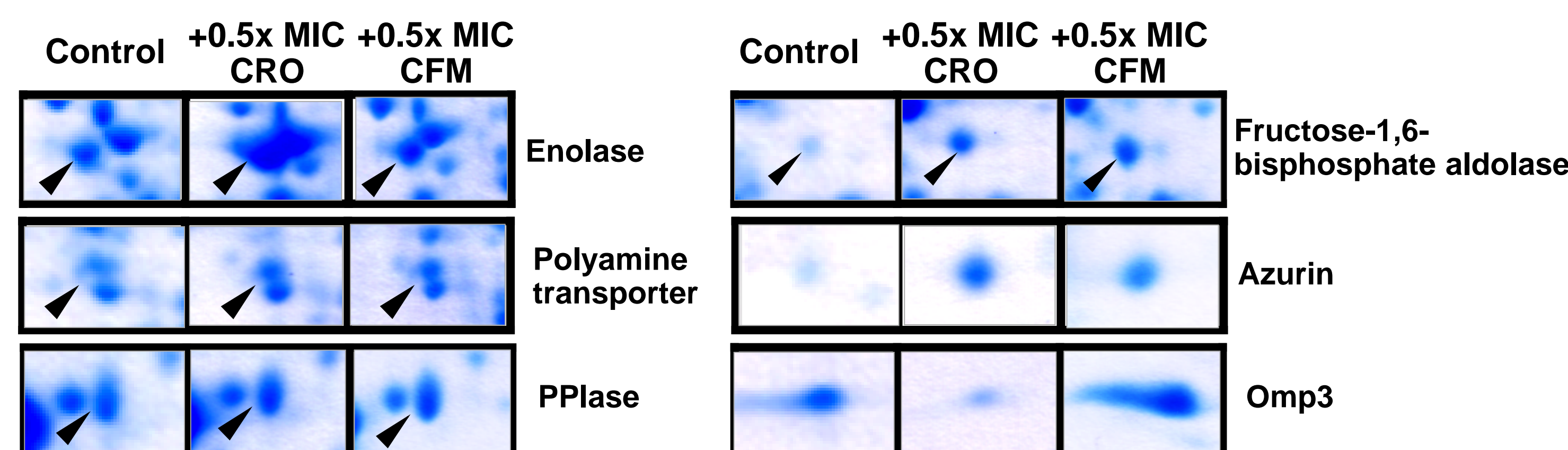


Figure 4. Enlarged partial 2-DE gels of some differentially expressed proteins after ESC exposure.

References:

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